Electrotransformation of *Streptococcus sanguis* Challis

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Abstract. Plasmid DNA was introduced into noncompetent cells of *Streptococcus sanguis* Challis by an electrotransformation technique. The procedure was simple and rapid, did not require elaborate pretreatment of cells, and yielded transformant colonies in 24 h. The maximum transformation efficiency attained was 2.1×10^4 transformants per μg of pVA736. Molecular rearrangements and deletions were not detected in plasmid DNA isolated from transformants.

Among streptococci, genetic transformation with chromosomal and plasmid DNA is restricted to species with a capacity for competence that permits the transport of DNA into the cell. Streptococcal species that may develop such a physiological state and thus are naturally transformable are not numerous and include strains of *Streptococcus pneumoniae* [2, 30, 38], *S. sanguis* Challis [17], group F Streptococcus sp [20], and *S. mutans* [15, 23, 31, 32].

The state of physiological competence in many strains is a transient and elusive property, influenced by the complexity of transforming media, pH, temperature, and the age of recipient cells [8, 41]. The induction of competence frequently requires elaborate and time-consuming manipulations of microbial populations, including isolation of single colonies, repeated passages in broth cultures, treatment with heat-inactivated horse serum, temperature shifts, and the harvesting of young, vigorously growing cultures between empirically defined optical density limits [3, 16, 19, 22, 31, 33, 34, 42].

In recent years, electroporation has become a widely used technique in the genetic transformation of plant protoplasts and animal cells [36]. In the electroporation process, cell membranes become reversibly permeabilized under the influence of high-field-strength electric pulses, permitting the uptake of DNA. Electroporation-dependent genetic transformation (electrotransformation) of various microorganisms has also been achieved [1, 5, 6, 12, 14, 24, 29, 37] including competence-negative streptococci without a natural transformation system such as *S. lactis* [9, 35], *S. cremoris* [21], and *S. thermophilus* [40].

Since *S. sanguis* Challis is probably the most extensively used streptococcal host system in the interspecies transfer and analysis of homologous and heterologous DNA of chromosomal or plasmid origin [3, 4, 10, 11, 13, 17–19, 25–28, 43] and its development of competence is not always reliable, it was of considerable interest to examine the efficiency of the electroporation-dependent transformation (electrotransformation) technique in this species. In this report we describe the electrotransformation of noncompetent cells of *S. sanguis* Challis with the erythromycin-resistance plasmids pVA736 and pAMβ1.

Materials and Methods

Bacterial cultures and maintenance conditions. *Streptococcus sanguis* Challis DL-1 was a gift from D. J. LeBlanc (University of Texas, San Antonio, Texas) and was routinely maintained in brain–heart infusion (BHI) or Todd-Hewitt (TH) broth. *S. thermophilus* ST128 was from our laboratory collection and maintained in Hogg–Jago–lactose (HJL) broth [39]. Erythromycin-resistant (Em^r) transformants of *S. thermophilus* ST128 were prepared with plasmids pVA736 [25] and pAMβ1 [7] according to a procedure reported earlier [40] and maintained in HJL broth with 20 μg/ml of erythromycin.

Plasmid DNA isolation. The transforming plasmids pVA736 and pAMβ1 were purified from deproteinized clear lysates of *S. thermophilus* ST128 cultures on ethidium bromide–cesium chloride density gradients as described [40]. Plasmid bands of the gradients were pooled, extracted with isopropanol three times, extensively dialyzed against 10 m*M* Tris–HCl–1 m*M* EDTA, pH 8.0, and sterilized by filtration prior to use.

Transformation of competent *S. sanguis* Challis. The development of competence in *S. sanguis* Challis DL-1 was facilitated by a procedure recommended by LeBlanc and Lee [19] with appro-

priate modifications. An overnight culture of *S. sanguis* Challis DL-1 grown in TH broth was serially diluted and plated on TH agar. After 48 h at 37°C, single colonies were picked and transferred six times in TH broth with incubation at 37°C in a 5% CO_2 atmosphere. Following the last passage, serial dilutions were made with TH broth supplemented with heat-inactivated horse serum at 1% (vol/vol) concentration. Incubation was continued for 16 h, and cultures showing an OD_{660} of 0.02–0.05 were used as host systems for transformation with pVA736 and pAMβ1. To 45 μl of cell suspension 1–5 μg of transforming DNA in 50 μl of 10 m*M* Tris–1 m*M* EDTA, pH 8.0, was added, and incubation was continued for 2 h at 37°C. Samples of undiluted and diluted transformation mixtures were spread on TH agar with 25 μg/ml of erythromycin. Plates were scored for Em^r colonies after 24–48 h at 37°C in 5% CO_2.

Electrotransformation of noncompetent *S. sanguis* Challis. A 16-h-old culture of *S. sanguis* Challis DL-1 was transferred to fresh TH broth (0.5% inoculum, vol/vol) and incubated at 37°C for 2–3 h or until OD_{660} of 0.25–0.3 was reached. Four ml of this culture was centrifuged and the pellet washed twice with 5 m*M* K_2HPO_4-KH_2PO_4 buffer, pH 7.0, before being dispersed in 1 ml of electroporation medium (EPM, 5 m*M* K_2HPO_4-KH_2PO_4 with 1 m*M* $MgCl_2 \cdot 6H_2O$ and 0.3 *M* raffinose, pH (4.5) [40].

To induce electroporation and transformation, an 0.85-ml aliquot of cell suspension in EPM was chilled to 4°C in a sterile cuvette before addition of 1.5 μg of transforming DNA (pVA736 or pAMβ1) in 50 μl of EPM. Electric pulses up to 6.25kV/cm were applied in 0.25kV/cm increments with a Bio-Rad Gene Pulser instrument set at 25 μF capacitance, with a single-hit pulsing regimen [40]. After electric pulsing, 0.1 volume of a 10× TH broth preparation was added, and the cell suspension was stored at room temperature for 2–3 h before aliquots were plated in 10 ml of 1.5% TH agar. After solidifying, this bottom layer was covered with 10 ml of 1.5% TH agar with 30 μg/ml of erythromycin. Plates were scored for Em^r transformants after 24–48 h of incubation at 37°C in a 5% CO_2 atmosphere.

To electrotransform post-log phase cell populations of noncompetent *S. sanguis* Challis, an overnight culture of strain DL-1 in TH broth was centrifuged, washed twice, and finally diluted to an OD_{660} of 0.25. A 4-ml aliquot of the cell suspension was prepared for electric pulsing as described above.

Analytical gel electrophoresis. Samples of plasmid DNAs isolated from randomly selected Em^r transformants of competent and noncompetent *S. sanguis* Challis were electrophoresed in Tris–boric acid–EDTA (pH 8.2) buffer in vertical 0.7% or 1% agarose gels, stained with 1 μg/ml of ethidium bromide, and photographed as described [39].

Materials. Microbiological media were products of Difco Laboratories*, Detroit, Michigan. The restriction endonuclease enzymes AvaI, EcoRI, HindIII, HpaII, and KpnI were from Bethesda Research Laboratories (Rockville, Maryland). All biochemicals and reagents were commercial products of the highest analytical purity. The Gene Pulser electroporation unit was purchased from Bio-Rad Laboratories (Richmond, California).

Table 1. Transformation of competent cells of *Streptococcus sanguis* Challis

Transforming plasmid	Number of transformants per microgram of DNA[a]
pVA736 (7.6kb)	2.3×10^6
pAMβ1 (26.5kb)	2.6×10^5

[a] Average values of three independent trials.

Results and Discussion

The results on the transformation of competent cell populations of *Streptococcus sanguis* Challis are shown in Table 1. The efficiency values obtained were in good agreement with values reported by others for the Em^r plasmids pGB301 and pDB101 [3], and the larger pAMβ1 [19]. Physiological competence, as expected, was a critical prerequisite for successful transformation by the conventional approach. When transformation was attempted with either 4-h- or 16-h-old noncompetent cultures of *S. sanguis* Challis grown in BHI or TH broth, no more than five Em^r transformants per microgram of DNA used were detected on selective agar plates.

In evaluating the electrotransformation technique as an alternative method for the direct introduction of DNA into intact cells, first the effect of voltage on culture survival was tested. Starting with a 10^8/ml initial cell count, there was no detectable loss of cell viability after the single-hit electric pulsing of noncompetent *S. sanguis* Challis cells between 1kV/cm and 5.62kV/cm. Cell death was noticed only when electric pulsing was conducted at 6.25kV/cm (maximum instrument output), resulting in approximately 80% reduction in viable cell count. The resistance of *S. sanguis* Challis to relatively high voltage settings was somewhat unexpected, since lethal effects of electric pulsing were observed in *S. cremoris* already at 3–3.75kV/cm [21], and loss of culture viability at 5kV/cm was found to be as high as 75% in the case of *S. thermophilus*, depending on the strain used [40]. Whether resistance to the voltages applied is an inherent physiological property of *S. sanguis* Challis or influenced by experimental conditions remains to be determined.

In the electrotransformation of noncompetent, early-log phase cells of *S. Sanguis* Challis, the number of Em^r transformants increased with increasing voltage and was at maximum at 5.62kV/cm for both transforming plasmids (Table 2). Because of its smaller molecular mass, pVA736 (7.6 kb) was taken

Table 2. Electrotransformation of noncompetent *Streptococcus sanguis* Challis

Voltage (kV/cm)	Transformation efficiency[a] with plasmid	
	pVA736	pAMβ1
2.5	0	0
3.12	6	0
3.75	10^2	8
4.37	1.2×10^3	1.1×10^2
5.0	1.4×10^4	4×10^2
5.62	2.1×10^4	8.5×10^2
6.25	1.9×10^4	6×10^2

[a] The transformation efficiency is defined as the number of transformants per microgram of DNA. Values represent averages of three independent trials.

up more efficiently than pAMβ1 (26.5 kb). This was not entirely unexpected, since similar results were found earlier in the electrotransformation of *S. thermophilus* with the same plasmids [40]. In other laboratories, electrotransformation of competence-negative streptococci with Em^r plasmids yielded transformation efficiency values ranging from 1.7×10^2 to 1.2×10^4 per microgram of DNA in *S. lactis* [9, 35], and up to 4×10^3 in *S. cremoris* [21], depending on the transforming plasmid used.

Post-log phase (16-h-old) cell populations of noncompetent *S. sanguis* Challis responded to electrotransformation in the same manner and transformed with pVA736 and pAMβ1 at efficiency levels identical with log-phase cultures (data not shown). This clearly indicated that culture age was not a significant factor in the electrotransformation of *S. sanguis* Challis.

Electrophoretic analysis of plasmids recovered from randomly selected Em^r transformants showed the presence of single entities with molecular masses of 7.6kb or 26.5kb, depending on the transforming plasmid used (Fig. 1). When plasmids isolated from ten Em^r/pVA736 transformants were analyzed, they displayed restriction endonuclease digestion patterns and fragment sizes identical with those reported by others for pVA736 [25]. This included single sites for EcoRI, HindIII, and KpnI, and two sites for both AvaI and HpaII. Apparently, the plasmid pVA736 did not undergo molecular rearrangements or deletions during the electrotransformation process, which underscores its potential as a cloning vector for streptococci.

Under the experimental conditions used, electrotransformation was somewhat less efficient than

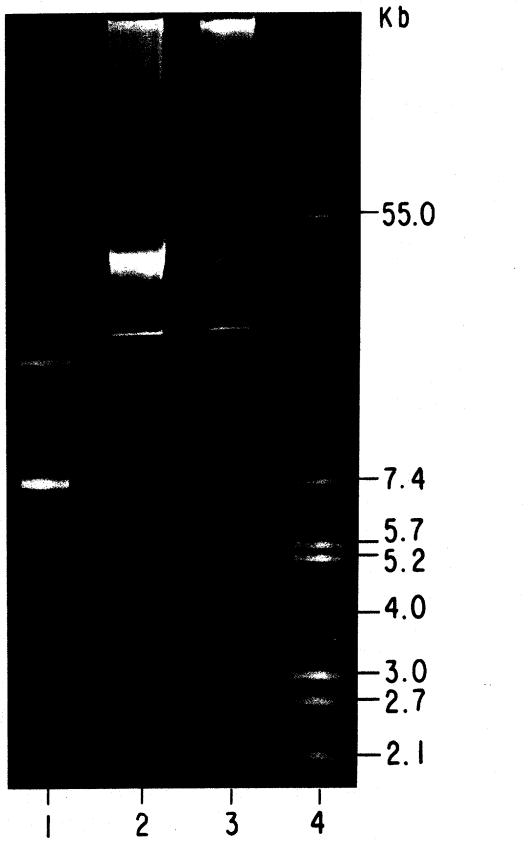

Fig. 1. Agarose gel electrophoresis of plasmids from Em^r transformants of *Streptococcus sanguis* Challis. Lanes: 1, pVA736 (ccc and oc forms, electrotransformation); 2, pAMβ1 (ccc and oc forms, electrotransformation); 3, pAMβ1 (ccc and oc forms, competence-dependent transformation); 4, *Escherichia coli* V517 plasmid standards.

competence-dependent transformation in the production of Em^r transformants of *S. sanguis* Challis. The maximum number of transformants obtained by electrotransformation was two to three orders of magnitude lower, depending on the transforming plasmid used. Thus, further refinements of conditions are necessary to improve the yield of transformants by electrotransformation.

Nevertheless, the electrotransformation technique represents distinct and significant advantages over the conventional transformation process. In the case of *S. sanguis* Challis, culture age, phase of the growth cycle, synthesis of soluble factors, pretreatment of cells, and various growth medium supplementations that add up to time-consuming processes in the induction of competence are no longer prerequisites for the successful uptake of DNA. Instead of the 2–4 days of the required pretransforma-

tion regimens, electrotransformation of noncompetent cells may be accomplished in a single day.

In conclusion, our results suggest that electrotransformation offers a simple and viable alternative to methods dependent on the inducibility of competence in *S. sanguis* Challis. by obviating the need for competent cells, electrotransformation will permit more convenient experimental designs and thus facilitate progress in genetic studies involving this important streptococcal host system.

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